I. THE INFLUENCE OF CERTAIN AMINO ACIDS ON CREATINE-CREATININE METABOLISM

II. THE ISOLATION OF THREONINE

BY

CHARLES ABRAM DEKKER

A. B., Calvin College, 1941

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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I. THE INFLUENCE OF CERTAIN AMINO ACIDS ON CREATINE-CREATININE METABOLISM

Introduction

The formation of creatine in the animal body is believed to be limited to the need of the cells for it. When the exact amino acid precursors of creatine were established (1, 2), it was considered necessary to make a final test of this hypothesis by simultaneously feeding glycine, arginine, and methionine, and observing the effect on creatine formation as reflected in the urinary excretion of creatine or creatinine. As an elaboration of this problem it was decided to feed both glycine, and a mixture of glycine and arginine, and to determine whether the production of either creatine or its intermediate, glycocyamine, was increased. Supplementary data on the fate of ingested creatine, creatinine, and glycocyamine were also sought in this study.

Experimental

Female rabbits were selected as experimental animals and were fed a basal diet of Purina Rabbit Chow Checkers, carrots, and oats. This was supplemented with one, two, or three of the amino acid precursors of creatine in amounts equivalent to 50 to 500 milligrams of creatinine. Twenty-four hour urine samples were collected and analyzed for total nitrogen, creatinine, total creatinine (creatine plus creatinine), and in some cases glycocyamine. Purified creatine, creatinine, and glycocyamine were also administered as supplements to the normal diet and similar determinations made. Feeding periods were usually four days in length.

Summary

Creatine was found to occur in significant amounts in the urine of all experimental animals while on the basal

diet. A high protein, low carbohydrate diet produced the highest creatine values, a phenomenon best explained as the result of an increased rate of cellular metabolism.

Supplementing the diet with glycine, arginine, methionine, or combinations of these amino acids, the precursors of creatine, furnished no evidence for increased creatine formation. The data did indicate, however, that arginine and glycine increase urinary glycocyamine.

Creatine ingestion for short periods gave rise to very little extra creatinine. This would be expected from the slow rate of conversion (2 per cent daily turnover). Further evidence for the irreversibility of the creatine to creatinine reaction *in vivo* was adduced from creatinine feeding experiments.

In a single study glycocyamine was confirmed as an intermediate in creatine production, its intake causing a slight but significant rise in creatine output.

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II. THE ISOLATION OF THREONINE

A. General Introduction

The procedure employed by McCoy, Meyer, and Rose (1) in the initial isolation of threonine has never been sufficiently improved to allow its preparation to be commercially practicable. Resolution of synthetic DL-threonine (2) is a

more advantageous method of production but is also uneconomical. Research on amino acid metabolism and the amino acid requirements of various mammals and microorganisms has increased the need for an improved procedure.

The excellent method of Shinn and Nicolet (3) for the determination of threonine and the advent of new reagents for the separation of amino acids from protein hydrolysates assured us that a more efficient process for threonine isolation was possible.

B. The Attempted Isolation of Threonine by the Use of Sulfonic Acids

Threonine-rich mixtures (20 to 40 per cent) of amino acids were treated with various sulfonic acids in 0.5 N. hydrochloric acid in an attempt to precipitate either threonine or any of the other amino acids contaminating the mixture. Neither 5-nitroanthraquinone-1-sulfonic acid, whose DL-threonine salt has a low solubility product, or any of the other sulfonic acids tried were successful in selectively precipitating threonine. In attempts to use the sulfonic acids for the removal of other amino acids in the mixture so as to improve the threonine concentration, only β-naphthalene-, 2,4-dinitro-1-naphthol-7-, and O-tosyl-p-phenolsulfonic acids were found to have any effect.

C. The Attempted Fractionation of Amino Acid Mixtures by the Use of Copper Salts

Town's (4) scheme for the fractionation of the amino acids on the basis of the solubilities of their copper salts seemed ideally suited for the concentration of threonine in our mixtures which were high in the water-soluble, methanol-soluble amino acids. However, we obtained no evidence that the copper salt of threonine was more insoluble in methanol than the copper salts of the other amino acids. The procedure was thus eliminated as a possible step in the isolation of threonine.

D. A New Procedure for the Isolation of Threonine

Introduction

Our attempts to find a specific precipitant for threonine having been unsuccessful, we were forced to obtain our product by gradually eliminating the other amino acids from a protein hydrolysate. If this could be accomplished without concurrent loss of large amounts of threonine, it would be possible to improve the yield of this protein constituent. New techniques and reagents in the field of amino acid isolation and separation appeared applicable to this work.

Experimental

The acidic, basic, and insoluble amino acids were removed from a fibrin hydrolysate with ion-exchange resins, and the effluent concentrated to small volume to precipitate the slightly soluble amino acids. Phenylalanine, glycine, alanine, and some other amino acids were partially removed from the filtrate as their 5-nitrobarbiturates, and the excess 5-nitrobarbituric acid was separated as the barium salt. Extraction of the resulting filtrate with butanol brought about further improvement of the threonine to nitrogen ratio of the water layer. The dry amino acids obtained from this layer were recrystallized from 80 per cent ethanol and extracted with glacial acetic acid. The insoluble material was recrystallized from 45 per cent ethanol to give crystalline threonine of 95 to 97 per cent purity. Yields of 15 to 20 per cent of the available threonine in fibrin were obtained.

Summary

A new procedure has been developed for the isolation of L-threonine from blood fibrin which achieves economies in time and labor.

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VITA

The author was born April 9, 1920, in Chicago, Illinois. He received his elementary and secondary education in the Christian school system of Grand Rapids, Michigan. He attended Calvin College, from which he received the Bachelor of Arts degree in June of 1941. The following September he entered the graduate school of the University of Illinois with an appointment as a Special Research Assistant in Chemistry, and held this position for the succeeding two years. From October, 1943, until June, 1944, he served as Teaching Assistant in Chemistry. He received a commission in the U. S. Naval Reserve in May, 1944, and served in the Asiatic-Pacific and American areas. After honorable release in July, 1946, he reentered the University of Illinois, and held appointments as Teaching Assistant in Chemistry from September, 1946, until June, 1947.